Diets of Three Species of Anurans from the Cache Creek Watershed, California, USA

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Abstract.—We evaluated the diets of three sympatric anuran species, the native Northern Pacific Treefrog, Pseudacris regilla, and Foothill Yellow-Legged Frog, Rana boylii, and the introduced American Bullfrog, Lithobates catesbeianus, based on stomach contents of frogs collected at 36 sites in 1997 and 1998. This investigation was part of a study of mercury bioaccumulation in the biota of the Cache Creek Watershed in north-central California, an area affected by mercury contamination from natural sources and abandoned mercury mines. We collected R. boylii at 22 sites, L. catesbeianus at 21 sites, and P. regilla at 13 sites. We collected both L. catesbeianus and R. boylii at nine sites and all three species at five sites. Pseudacris regilla had the least aquatic diet (100% of the samples had terrestrial prey vs. 5% with aquatic prey), followed by R. boylii (98% terrestrial, 28% aquatic), and L. catesbeianus, which had similar percentages of terrestrial (81%) and aquatic prey (74%). Observed predation by L. catesbeianus on R. boylii may indicate that interaction between these two species is significant. Based on their widespread abundance and their preference for aquatic foods, we suggest that, where present, L. catesbeianus should be the species of choice for all lethal biomonitoring of mercury in amphibians.

During 1997–98, we conducted a study to evaluate levels of mercury bioaccumulation in macroinvertebrates, fish (Schwarzbach et al., 2001), insectivorous birds (Hothem et al., 2008), and amphibians (Hothem et al., in press) in the Cache Creek Watershed, an area with a long history of mercury contamination from mining and natural sources (Rytuba, 2000). Within the aquatic ecosystem, inorganic mercury may be converted to the more toxic form, methylmercury, a neurotoxin that is readily accumulated by aquatic organisms (Ullrich et al., 2001). The primary objective of the original study was to relate mercury accumulation by biota within the Cache Creek Watershed to sources of mercury.

A secondary objective was to evaluate anurans as potential bioindicators of mercury contamination. An evaluation of dietary preferences of the resident anurans was essential to explain interspecific differences in mercury bioaccumulation and to determine which species might serve as the best bioindicator of mercury contamination in the aquatic ecosystem.

Of the three species evaluated in this study, the only nonnative was the American Bullfrog, Lithobates catesbeianus, a species known to feed on a wide variety of aquatic prey, including adult and larval amphibians (Bury and Whelan, 1984). Lithobates catesbeianus has greatly expanded its range since its original introduction to California in 1896 (Heard, 1904; Jennings and Hayes, 1985) and is now present in suitable habitat throughout the state (Jennings, 1996; Stebbins, 2003), including the Cache Creek Watershed. *Lithobates catesbeianus* is potentially competing with all life stages of two native residents of the watershed, the Foothill Yellow-Legged Frog, Rana boylii (Kupferberg, 1997) and the Northern Pacific Treefrog, Pseudacris regilla (Govindarajulu et al., 2006).

Rana boylii is a California species of special concern (Jennings, 2004) and occurs in much of the northern half of California west of the Cascade crest and Sierras from sea level to

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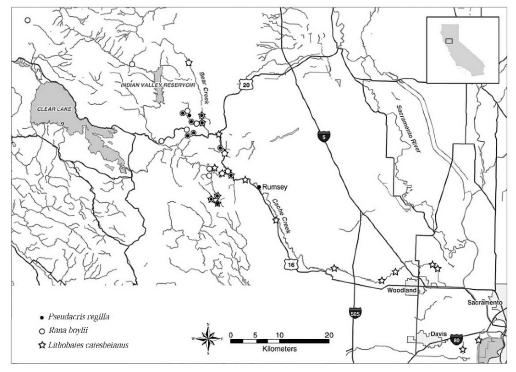


Fig. 1. Sites within the Cache Creek Watershed sampled for amphibians during 1997-98.

about 1,830 m (Livezey, 1962; Stebbins, 2003). Habitat alterations have contributed to reductions in the range of *R. boylii* (Jennings and Hayes, 1994), but competition with and predation by *L. catesbeianus* may be contributing to these reductions (Moyle, 1973; Kupferberg, 1997; Crayon, 1998). The range of *P. regilla* includes the grasslands, chaparral, woodland, and agricultural areas of Northern California, from sea level to over 3,500 m (Brattstrom and Warren, 1955; Jennings, 2000). *Pseudacris regilla* is common in the Cache Creek Watershed, and its range overlaps those of both of the other species.

The diet of *L. catesbeianus* has been widely studied and found to be diverse but mostly dominated by aquatic prey (Werner et al., 1995; Cross and Gerstenberger, 2002; Hirai, 2004). Based primarily on anecdotal information in a few published studies, the diet of *R. boylii* appears to be more terrestrial than that of *L. catesbeianus* (Fitch, 1936; Van Wagner, 1996). Studies of *P. regilla* have shown that they feed predominately on flying insects (Needham, 1924; Brattstrom and Warren, 1955; Johnson and Bury, 1965). However, little is known about how diets of these three species compare within the same watershed.

Stomach contents of these three sympatric species collected from similar habitats within

the same watershed were analyzed to enable us to estimate dietary preferences. Information on the aquatic and terrestrial make-up of the three anurans' diets will help determine the preferred species for use as a bioindicator of mercury contamination in the aquatic ecosystem.

Materials and Methods

Study Site.—The 2,950 km² Cache Creek Watershed is located in the North Coast Range of California, about 130 km north of San Francisco; Cache Creek flows into the Sacramento River just northeast of the city of Woodland (Fig. 1). The watershed is located primarily in Lake, Colusa, and Yolo Counties but also extends into parts of Napa, Mendocino, and Sonoma Counties. It is composed of three distinct subbasins: the North Fork of Cache Creek, the South Fork of Cache Creek, and Bear Creek.

Collection sites included reference sites upstream from mercury mines, sites within the mercury mining area, and sites that were immediately downstream of mines (Fig. 1). Also included were sites located within the high-gradient Cache Creek Canyon further downstream from the mines and sites within the low-gradient portions of Cache Creek, which extends from the Cache Creek Canyon just upstream of the town of Rumsey through a

gravel-mining area to the Cache Creek Settling Basin near the Sacramento River. *Lithobates catesbeianus* was also collected from the Yolo Basin Wildlife Area, located about 15 km downstream of Cache Creek in the Yolo Bypass. Specific collection sites were selected based on knowledge of potential mercury contamination, site accessibility, and the presence of appropriate study organisms. Samples were collected from March to July 1997 and March to September 1998.

Sample Collections.—Frog specimens were collected by hand or with a dip net during the day, or by hand or with a gig and spotlight after dark, and placed in a plastic bag on wet ice. For each specimen, we recorded the site, species, date, time, and collector and attached this information to the specimen or its container. At the laboratory, frogs were humanely euthanized with MS-222 the same day they were collected and kept frozen until they could be processed within two days after collection. After the carcass was thawed, we measured the snout-vent length (SVL) with calipers (0.01 mm) and determined the mass of the carcass using an electronic balance (± 0.01 g). We dissected each specimen, determined the sex by directly examining the gonads, and removed the digestive tract. The stomach contents were preserved in 70% ethyl alcohol for later identification, and the carcasses were stored frozen pending mercury analysis.

Prey Items.—Prey items found in each digestive tract were classified to the lowest possible taxonomic level (order or family) using appropriate keys and a binocular microscope. In addition, we classified each prey type as aquatic, terrestrial, or both, depending on its stage of development and the habitat in which it occurred. Larval aquatic insects were classified as aquatic, whereas flying adults were considered terrestrial. We used these classifications to evaluate the sampled species for their suitability to serve as biomonitors of mercury contamination, assuming that a more aquatic diet would more likely reflect mercury contamination in the sampled area.

Data Analysis.—With the exception of the frogs of unknown sex and the four juvenile *R. boylii*, all frogs were included in calculations of mean mass and mean SVL. We used two-sample *t*-tests for normal data to determine whether there were gender differences in mass or SVL for each species. Where tests of normality or equal variance failed, we used the Mann-Whitney Rank Sum Test (*T*-statistic defined as the sum of the ranks in the smaller sample; SigmaStat 3.1, SYSTAT Software, Inc., Point Richmond, CA).

Digestive tracts (esophagi and stomachs) of 107 L. catesbeianus (57 female, 46 males, four unknown sex), 65 R. boylii (38 females, 27 males), and 54 P. regilla (nine females, 44 males, and one unknown sex) were examined. Thirtyone of the *L. catesbeianus* and four *R. boylii* were juveniles. Too few juvenile R. boylii were examined, and they were not included in the statistical analyses. Diets of juvenile and adult *L*. catesbeianus were compared, but only adults were used in interspecific comparisons (Fig. 2). The frogs that contained no identifiable prey items (seven *L. catesbeianus*, nine *R. boylii*, and 31 P. regilla) and the four L. catesbeianus of unknown sex were not included in any comparisons. Because of small sample size (N <100), we used Fisher's Exact Test to detect diet differences between sexes and species, based on presence or absence of each taxon.

A total of 65 adult L. catesbeianus (34 female, 31 males), 31 juvenile L. catesbeianus (19 females, 12 males), 52 adult R. boylii (30 females, 22 males), and 23 adult P. regilla (three females, 20 males) was included in the analysis of frequency of occurrence (Table 1). Only adults were used in comparisons between species (Fig. 2); the nine sites below bridge 22–19 and the two reference sites from the far northwest part of the Cache Creek Watershed (Fig. 1) were not included because prey availability was not comparable to the other sites. We used Fisher's Exact Test to compare aquatic and terrestrial invertebrate prey types. Adult amphibians used in the species comparisons were included only if prey could be identified to a low enough level to be categorized as either aquatic or terrestrial.

RESULTS

Habitat Distribution of Species.—We collected individuals of one or more anuran species (adult P. regilla, adult and juvenile R. boylii, and L. catesbeianus) from 23 sites from March to July 1997 and 19 sites from March to September 1998 (Fig. 1). Six of the 36 sites were sampled both years. Although P. regilla likely ranged throughout the watershed, they were only collected at 13 sites, all in the upper parts of the watershed, at and upstream of the Rumsey bridge. We collected R. boylii from 22 of the 26 sites located at or upstream of the confluence of the Cache and Bear Creeks (about elevation 190 m). Nine of those 22 sites also had L. catesbeianus present. Overall, L. catesbeianus were collected from 21 sites, which were mostly perennial streams; L. catesbeianus was the only species collected at the nine sites in the low stream gradient region of Cache Creek downstream of bridge 22-19, located about 4.1 km northwest of Rumsey (elevation 155 m) to the

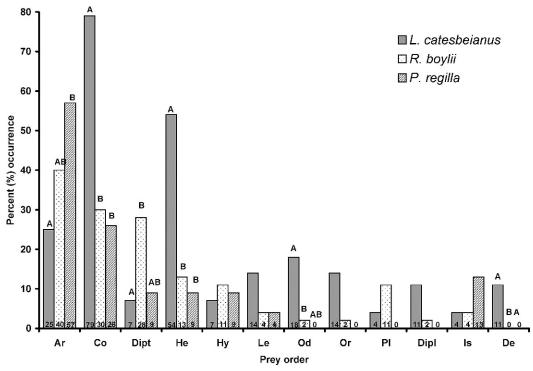


Fig. 2. Percent occurrence (shown along the x-axis) of invertebrate prey orders found in *Lithobates catesbeianus* (N=28), *Rana boylii* (N=47), and *Pseudacris regilla* (N=23) collected from Cache Creek Watershed, California, 1997–98. Prey orders are as follows: Ar = Araneae; Co = Coleoptera; Dipt = Diptera; He = Hemiptera; Hy = Hymenoptera; Le = Lepidoptera; Od = Odonata; Or = Orthoptera; Pl = Plecoptera; Dipl = Diplopoda; Is = Isopoda; De = Decapoda. Interspecific differences for individual prey orders are significant if labeled with different letters; orders with no labels were not significantly different.

Yolo Basin Wildlife Area (elevation 5 m). We collected all three species at five of the 36 sites.

Species Characteristics.—For L. catesbeianus, there were no differences in mass based on gender for adults (Mann-Whitney T = 1079; n1 = 34; n2 = 38; P = 0.069) or for juveniles ($t_{29} =$ 0.815; P = 0.422). Differences in SVL based on gender were not significant for adults (t_{70} = 1.743; P = 0.086) or for juveniles (Mann-Whitney T = 214; n1 = 12; n2 = 19; P = 0.383; Table 1). However, the masses of adult female R. boylii (Mann-Whitney T = 539; n1 = 27; n2 = 10034; P < 0.001) and P. regilla (Mann-Whitney T =395; n1 = 9; n2 = 44; P < 0.001) were significantly greater than the masses of males. The SVLs of the adult female *R. boylii* ($t_{59} = 5.33$; P < 0.001) and P. regilla (Mann-Whitney T =375; n1 = 9; n2 = 44; P = 0.002) were also significantly greater than the SVLs of the males (Table 1).

Diet Composition.—Certain prey items dominated the diets of the three sympatric anurans included in this study. Spiders (Order Araneae) were more commonly detected in juvenile female *L. catesbeianus* than in adult females

(Fisher's Exact Test; P = 0.005), and, overall, spiders were found in more juvenile than (55%) adult L. catesbeianus (23%; P = 0.003; Table 1). Spiders were the most commonly found prey in R. boylii and P. regilla, with significantly more found in P. regilla than in L. catesbeianus (P =0.042; Fig. 2). Insects (Class Insecta) were found in significantly more stomachs of adult male L. catesbeianus than in those of adult females (P =0.046). Beetles (Order Coleoptera), both aquatic (primarily Hydrophilidae) and terrestrial (primarily Carabidae), were the most common prey items found in L. catesbeianus (Table 1), and beetles were detected in more L. catesbeianus stomachs than in either *P. regilla* or *R. boylii* (*P* < 0.01; Fig. 2). Dipterans were more commonly found in R. boylii than in L. catesbeianus (P = 0.039), whereas the occurrence of dipterans in P. regilla was not different from either of the other species (Fig. 2). Significantly more hemipterans (especially water striders, family Gerridae) were detected in L. catesbeianus than in either of the other two species. More odonates (primarily dragonfly larvae, family Gomphidae) were detected in L. catesbeianus, but the differences were only significant when compared with *R. boylii* (Fig. 2). Stoneflies (Order Plecoptera), strictly aquatic in the larval form, were only detected in *R. boylii* and *L. catesbeianus*, whereas pillbugs, a strictly terrestrial isopod, were detected more often in *P. regilla* (Table 1, Fig. 2).

Louisiana Red Swamp Crayfish (Order Decapoda; *Procambarus clarkii*) were only detected in *L. catesbeianus*. Overall, a higher percentage of the female adults contained crayfish than did the males (P=0.022; Table 1). However, this comparison was likely biased because 32.4% of the females but only 9.7% of the males collected in this study were from the Yolo Basin Wildlife Area, an area with apparently higher populations of crayfish than the other sites and an area where 93% of the *L. catesbeianus* contained one or more crayfish.

Vertebrates were primarily detected in the stomachs of *L. catesbeianus*, but identification was often difficult based on the advanced degree of digestion. Vertebrates included six unidentifiable fish, one *P. regilla*, parts of two unidentified frogs (likely *R. boylii*), one Southern Alligator Lizard (*Elgaria multicarinata*), one unidentified snake, two hatchling Western Pond Turtles (*Actinemys marmorata*), feathers (in two stomachs), and unidentified mammalian hair and bones (in three stomachs; Table 1). Although most of the vertebrates were detected in the stomachs of *L. catesbeianus*, evidence of a mammal (hair and bones) was found in the stomach of one *R. boylii*.

Unidentified plant material was found in 26.0% of the stomachs of *L. catesbeianus* (Table 1). Only 3.8% of the *R. boylii*, and none of the *P. regilla*, contained plant material. Gravel was found in nearly one-third (31.3%) of the *L. catesbeianus* stomachs that were evaluated (Table 1), a far higher percentage than was found in either *R. boylii* (5.8%) or *P. regilla* (4.3%).

Based on identifiable prey, there were no differences between sexes in the presence of terrestrial or aquatic prey for any of the three species of anurans. Therefore, the sexes were combined for comparisons among species. Lithobates catesbeianus had significantly fewer stomachs (81.5%) with one or more item of terrestrial origin than did R. boylii (97.5%; P = 0.035). Differences in percentage terrestrial prey between L. catesbeianus and P. regilla were not significant (P = 0.059), likely related to the small P. regilla sample size. Rana boylii and P. regilla were not different in terrestrial prey presence (P = 1.00), with all but one stomach containing at least one terrestrial item. However, comparisons of aquatic prey occurrence in the three species were significant (P < 0.05) for all species combinations, with L. catesbeianus having the highest percentage of stomachs with aquatic prey (74.1%), followed by *R. boylii* (27.5%), and *P. regilla* (4.8%).

DISCUSSION

In areas contaminated with mercury, fish are commonly used to assess ecological and human health, to provide data on contaminant trends, and to examine a wide range of natural and anthropogenic stressors (e.g., Parks et al., 1991; Goulet et al., 2008). Fish are suitable for identifying and evaluating the bioavailability of mercury sources because they normally occupy an upper trophic level in the food web, bioaccumulate mercury, and often have restricted home ranges (Peterson et al., 1996). However, in certain habitats, such as intermittent streams, ponds, and mine tunnels, fish may not be available, and amphibians may be the only readily available taxon that meets the biomonitoring criteria. Because the aquatic food web is the most likely route of mercury contamination (Ullrich et al., 2001), the species with the most aquatic food habits is usually the preferred choice to monitor mercury contamination.

Since its original introduction to California over 100 years ago, the range of *L. catesbeianus* has extended into the Cache Creek Watershed. We found *L. catesbeianus* at 58% of our sample sites, including 100% of the nine downstream sites (Fig. 1), an area outside the historic range of *R. boylii*. Our collections indicate that the two ranid species co-occurred at no less than 33% (nine of 27) of the sites sampled upstream from Rumsey and that all three species co-occurred at five sites, but we did not estimate population status or trends for the three species.

Lithobates catesbeianus consume a wide variety of invertebrate (Table 1) as well as vertebrate prey, including fish, lizards, snakes, turtles, and other anurans (Frost, 1935; Bury and Whelan, 1984; Wylie et al. 2003; Table 1). In this study, gravel and vegetation also were found in the L. catesbeianus stomachs (Table 1). Although the vegetation was most likely ingested accidentally (Korschgen and Moyle, 1955), the ingestion of gravel may be accidental or may actually aid in digestion and breakdown of food (Evans and Lampo, 1996). A small percentage of R. boylii and P. regilla also contained gravel, and two female *R. boylii* contained vegetation, but, unlike L. catesbeianus, neither species showed any evidence of predation on other anurans. We found remains of three anurans in the L. catesbeianus digestive tracts (Table 1), and a male *L. catesbeianus* collected from Davis Creek, a tributary to Cache Creek, but not included in this tabulation, contained two adult R. boylii (Crayon, 1998). The identification of postmeta-

Table 1. The percent frequency of occurrence of aquatic (a), terrestrial (t), and both aquatic and terrestrial (b) prey taxa found in stomachs and esophagi of male (M) and female (F) adult (ad) and juvenile (jv) *Lithobates catesbeianus* and adult *Rana boylii* and *Pseudacris regilla* from the Cache Creek Watershed, California, 1997–98. Percent occurrence for Class and Order include those that were not identified to the lower taxon listed.

		L. catesbeianus				R. boylii		P. regilla	
Sex/age Number of frogs Mean SVL (mm)		M/ad 31 130.7	M/jv 12 101.4	F/ad 34 139.4	F/jv 19 90.9	M/ad 22 43.9*	F/ad 30 54.4*	M/ad 20 32.4*	F/ad 3 37.0*
Mean mass (g)		246.9	112.0	297.0	94.9	11.2*	22.0*	2.98*	5.57*
Class Order Family	Prey type a/t/b								
Arachnida	t	32	58	15°	53°	41	43	63	33
Araneae	t	32	58	15°	53°	41	40	63	33
Opiliones	t	0	0	0	0	0	3	0	0
Insecta	b	87*	83	65*	74	73	73	47	67
Coleoptera	b	65	67	56	42	27	27	25	33
Carabidae	t	29	17	15	21	5	10	15	0
Cicindelidae Curculionidae	t	6 0	8 0	3 3	0 5	0	0	0 5	0
Dytiscidae	t a	0	0	0	0	9	0	5	0
Hydrophilidae	a	19	25	18	11	5	0	0	0
Staphylinidae	t	0	0	0	0	5	7	0	0
Tenebrionidae	t	0	0	6	0	0	0	0	0
Dermaptera Forficulidae	t t	0	0	6 6	0	9 9	3 0	0	0
Diptera	t	6	17	0	0	27	23	10	0
Chironomidae	a	0	0	0	0	0	3	0	0
Culicidae	t	0	0	0	0	0	3	0	0
Dolichopodidae	t .	0	0	0	0	0	7	0	0
Dryomyzidae Empididae	t t	0	8	0	0	0 5	0	0	0
Tipulidae	t	0	0	0	0	0	3	5	0
Hemiptera	b	35	42	32	47	23	7	10	0
Belostomatidae	a	3	8	0	5	0	0	0	0
Naucoridae	a	6	8	6	21	0	0	0	0
Nepidae	a	3	0	6	0	0	0	0	0
Gerridae	a	23	17	21	0	14	3	0	0
Gelastocoridae Saldidae	a	0	8	0	5 0	0 9	0	0	0
Veliidae	t a	6	0	0	0	0	3	0	0
Hymenoptera	t	6	8	0	5	5	17	10	0
Formicidae	t	0	0	0	5	5	7	5	0
Isoptera	t	0	0	0	0	0	0	5	0
Lepidoptera	t	10	17	15	16	0	10	0	33
Noctuidae	t	0	0	9	0	0	3	0	33
Pyralidae	t	0	8	0	0	0	7	0	0
Megaloptera Corydalidae	a a	0	0	0	0	0	3 3	0	0
Odonata	a	19	33	9	16	0	3	0	0
Coenagrionidae Gomphidae	a a	3	0 8	0	0	0 0	0 0	0	0
Orthoptera	t	23	33	18	5	0	7	0	0
Gryllacrididae	t	0	8	0	0	0	0	0	0
Gryllidae	t	3	8	6	5	0	0	0	0
Stenopelmatinae	t	0	0	3	0	0	0	0	0
Plecoptera	a	0	8	3	0	9	10	0	0
Tricoptera	a	3	8	0	11	0	0	0	0
Hydropsychidae	a	0	0	0	5	0	0	0	0
Chilopoda	t	6	0	3	0	0	3	0	0

Table 1. Continued.

		L. catesbeianus				R. boylii		P. regilla	
Sex/age Number of frogs Mean SVL (mm) Mean mass (g)		M/ad 31 130.7 246.9	M/jv 12 101.4 112.0	F/ad 34 139.4 297.0	F/jv 19 90.9 94.9	M/ad 22 43.9* 11.2*	F/ad 30 54.4* 22.0*	M/ad 20 32.4* 2.98*	F/ad 3 37.0* 5.57*
Class Order Family	Prey type a/t/b								
Geophilomorpha	t	6	0	3	0	0	3	0	0
Diplopoda	t	6	0	3	5	5	0	0	0
Glomerida Chordeumatida	t t	3 3	0	0 3	5 0	5 0	0	0	0 0
Melacostraca	b	19	0	38	11	5	3	10	33
Isopoda Decapoda	t a	6 13*	0	0 38*	0 11	5 0	3 0	10 0	33 0
Actinopterygii Amphibia	a b	3 3	17 0	6 6	5 0	0	0	0	0
Anura	b	3	0	6	0	0	0	0	0
Reptilia	b	0	8	3	0	0	0	0	0
Squamata Anguidae Colubridae	b t b	0 0 0	8 0 8	3 3 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Testudines Emydidae	a a	3 3	0	0	5 5	0	0	0	0
Aves (birds) Mammalia (hair and bones) Gravel Plant material	t b - -	0 0 29 29	0 8 25 25	3 3 38 26	5 0 26 21	0 5 0	0 0 10 7	0 0 5 0	0 0 0 0

^{*}Significant difference between sexes within species ($P \le 0.05$).

morphic native anurans in the digestive tracts of sympatric *L. catesbeianus* is conclusive evidence that predation is occurring, but we did not evaluate the impact of *L. catesbeianus* on the native populations.

In a study of a watershed invasion by *L. catesbeianus*, Kupferberg (1997) reported that larval competition may explain reduced *R. boylii* populations where *L. catesbeianus* are well established (Kupferberg, 1997). Because of its susceptibility to population declines, and because it is a species of special concern (Jennings, 2004), *R. boylii* would not be the most appropriate species for biomonitoring in the Cache Creek Watershed. Although *P. regilla* is abundant and is not a species of special concern, it is not suitable as a biomonitor of mercury contamination in streams based on its primarily terrestrial diet (this study).

Results from this study show that a high percentage of the stomachs of all three species had one or more terrestrial prey item, but the species with the highest percentage of stomachs with aquatic prey was *L. catesbeianus*. In addition to having the most aquatic diet of the

three species sampled in this study, it was not surprising that *L. catesbeianus* also exhibited the highest mercury concentrations (Hothem et al., in press), especially when compared with the other species at the same sites. Furthermore, unlike *R. boylii* and *P. regilla*, *L. catesbeianus* is an introduced species in California, often competing with natives. Therefore, we recommend that whenever lethal monitoring of amphibians for mercury is required, the first choice should be *L. catesbeianus*, replacing the use of native and special-status amphibians as biomonitors in all but the most unusual circumstances.

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[°] Significant difference between age groups ($P \le 0.05$).

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